DRUG-INDUCED HEMOLYTIC ANEMIAS AND CONGENITAL GALACTOSEMIA*

EXAMPLES OF GENETICALLY DETERMINED DEFECTS IN ERYTHROCYTE METABOLISM

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THIS discussion of "in-born errors" of erythrocyte metabolism is to be limited to a consideration of drug-sensitive hemolytic anemias and congenital galactosemia. Recent studies have established that both of these clinical syndromes are associated with specific erythrocyte enzymatic deficiencies.

DRUG-SENSITIVE HEMOLYTIC ANEMIAS

In 1948, Earle[1] observed the occurrence of hemolytic anemia in five to ten per cent of American Negro troops who had received pamaquine in the therapy of malaria. Similar experiences were reported following the use of primaquine[2]. These hemolytic episodes were observed very rarely among American Caucasian troops. Alving and his co-workers[3], studying the mechanism of primaquine-induced hemolytic anemia, observed that the hemolysis is self-limited. The lysis of red cells is a function of cell age, the older cells being the more susceptible[4]. Red cells of sensitive individuals are normal morphologically and have a normal life span unless challenged by a suitable agent[5]. These cells have no detectable abnormal antibodies and no disturbance either in osmotic or mechanical fragility[5].

Defects in Erythrocyte Metabolism. The erythrocytes of drug-sensitive subjects do have intrinsic metabolic defects. Figure 1 illustrates certain of the reactions of erythrocyte metabolism which are affected, either directly or indirectly, by the biochemical abnormalities in red cells of these subjects. Erythrocyte glucose utilization involves the phos-

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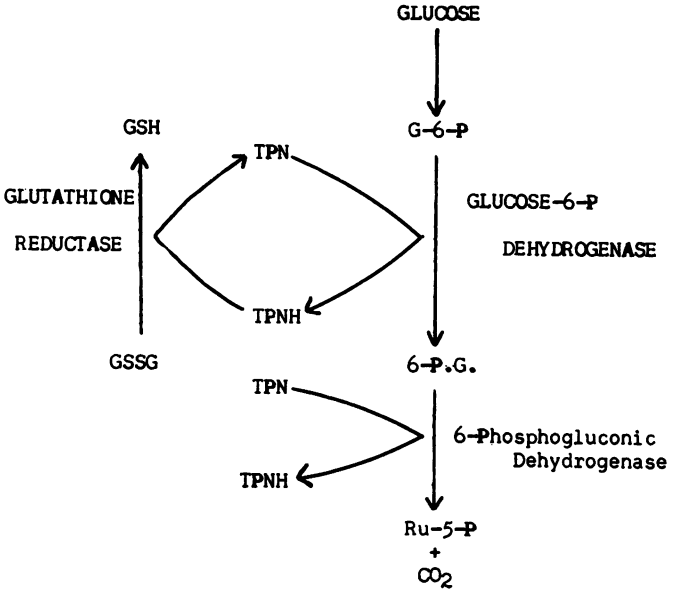

Fig. I—Schematic representation of certain reactions of erythrocyte metabolism which are affected by the defect present in red cells of subjects with a genetically determined predisposition to drug-induced hemolytic anemias. Abbreviations employed: G-6-P, glucose-6-phosphate; 6-P.G., 6-phosphogluconic acid; Ru-5-P, ribulose-5-phosphate; TPN, triphosphopyridine nucleotide; TPNH, reduced triphosphopyridine nucleotide; GSSH, oxidized glutathione; GSH, reduced glutathione.

phorylation of glucose to glucose-6-phosphate which in turn may be metabolized either via the reactions of anaerobic glycolysis or of the pentose phosphate pathway[6, 7]. The first of the oxidative reactions of the pentose phosphate pathway results in the conversion of glucose-6-phosphate to 6-phosphogluconic acid. This reaction is catalyzed by the enzyme glucose-6-phosphate dehydrogenase, requires as a co-factor, triphosphopyridine nucleotide (TPN) and generates reduced TPN (TPNH). 6-Phosphogluconic acid is then oxidized to the five carbon sugar, ribulose-5-phosphate. In this reaction, another molecule of TPN is reduced to TPNH and CO_2 is generated by the oxidation of the first carbon of glucose. These reactions represent the only pathway in mature erythrocytes for the production of reduced TPN and for the oxidation of glucose to CO_2[8, 9]. Red cells possess the enzyme glutathione

reductase which catalyzes the conversion of glutathione (GSSG) to reduced glutathione (GSH). This reaction requires as a co-factor, TPNH, and results in the generation of TPN[10].

The concentration of reduced glutathione in erythrocytes of drug-sensitive subjects is generally lower than that of normal persons[11], though there is considerable overlapping of values[12, 13]. Beutler[14] has described a "reduced glutathione stability test" which permits the detection of primaquine-sensitive individuals by an *in vitro* assay. This test is based on the finding that following the *in vitro* incubation of red cells with acetylphenylhydrazine, erythrocytes from drug-sensitive, but not from normal, subjects show a marked decrease in the concentration of reduced glutathione. Carson and his co-workers[15] demonstrated that the erythrocytes of drug-sensitive persons are deficient in the enzyme glucose-6-phosphate dehydrogenase. This finding of deficiency in red cell dehydrogenase was confirmed[16, 17] and found to be associated with a reduced capacity of these erythrocytes to oxidize glucose to CO_2, as well as a lowered rate of oxygen consumption when incubated in the presence of methylene blue[9]. Schrier and his co-workers reported that these erythrocytes have increased concentrations of the enzymes, glutathione reductase[18] and aldolase[19] and of TPN[20].

The most marked biochemical deviation from normal in the red cells from sensitive subjects appears to be the decrease in glucose-6-phosphate dehydrogenase. The other alterations in the metabolism of these erythrocytes could be secondary to the dehydrogenase deficiency. Thus, the reduction in glucose-6-phosphate dehydrogenase might be associated with a decreased rate of TPNH formation. A deficiency in TPNH would limit the rate of GSH generation and could account for the apparent instability of reduced glutathione. It has been suggested that the increase in glutathione reductase activity may represent a compensatory phenomenon, providing for increased concentrations of TPN and for more efficient GSH formation[12].

Substances Which May Induce Hemolytic Anemias. The elucidation of these biochemical abnormalities in erythrocytes of drug-sensitive subjects has not provided an explanation of the mechanism of the increased hemolysis following the ingestion of various agents. The list of such agents associated with hemolytic anemia is ever increasing and now includes nitrofurantoin[21], sulfanilamide[22, 23], acetanilid[22], various vitamin K substitutes[24], phenacetin[22], naphthaline[16, 25], fava bean[16, 17, 27, 28]

and isolated cases have been observed associated with the ingestion of para-amino salicylic acid, isioniazid and salicylic acid[29, 30].

Nitrofurantoin[21], certain vitamin K analogues and the naphthaline metabolites, γ and β naphthol[24], have been demonstrated *in vitro* to lead to a decrease in the reduced glutathione concentration but not hemolysis of erythrocytes from drug-sensitive individuals. However, sulfonamides and various preparations of the fava bean have not been shown to have similar *in vitro* effects[29]. Alving and his co-workers[12] have reported that administration of 30 mg. primaquine base (twice the daily therapeutic dose for vivax malaria) causes hemolysis in essentially all subjects with drug-sensitive erythrocytes. It should be emphasized, however, that subjects with deficient erythrocyte glucose-6-phosphate dehydrogenase may ingest certain of these agents without any apparent ill effects[29, 30]. In addition, patients who are unaffected by fava bean ingestion developed hemolytic anemia associated with sulfonamide administration[23]. The occurrence of increased hemolysis may in part be related to the dose of the potentially offending agent. In addition, different mechanisms may be involved in the hemolytic anemias induced by the different agents. It may be speculated that erythrocytes deficient in TPNH generation may be particularly susceptible to hemolysis because of reduced capacity to detoxify drug products or because of deficient synthetic processes, such as membrane lipid synthesis[6, 7].

Effect of Age on Erythrocyte Enzyme Activity. It is possible that some insight has been gained into a curious clinical aspect of the drug-induced hemolytic anemias, namely, their tendency to be self-limited and a function of cell age. It has been demonstrated in observations on normal subjects, that glucose-6-phosphate dehydrogenase activity is high in young erythrocytes and decreases markedly with the *in vivo* aging of these cells[31]. Studies in subjects with erythrocyte glucose-6-phosphate dehydrogenase deficiency have revealed that the level of this enzyme is higher in young, compared with old red cells[32]. The enzyme has been assayed in red cells of control subjects and persons whose erythrocytes had intermediate (2.5 to 4 standard deviations below the mean value for the control group) and low (less than 4 standard deviations below the mean for the control group) glucose-6-phosphate dehydrogenase activities. The whole erythrocyte population was separated into fractions of relatively young and old mean cell age by a method of serial osmotic hemolysis[33]. In subjects whose whole

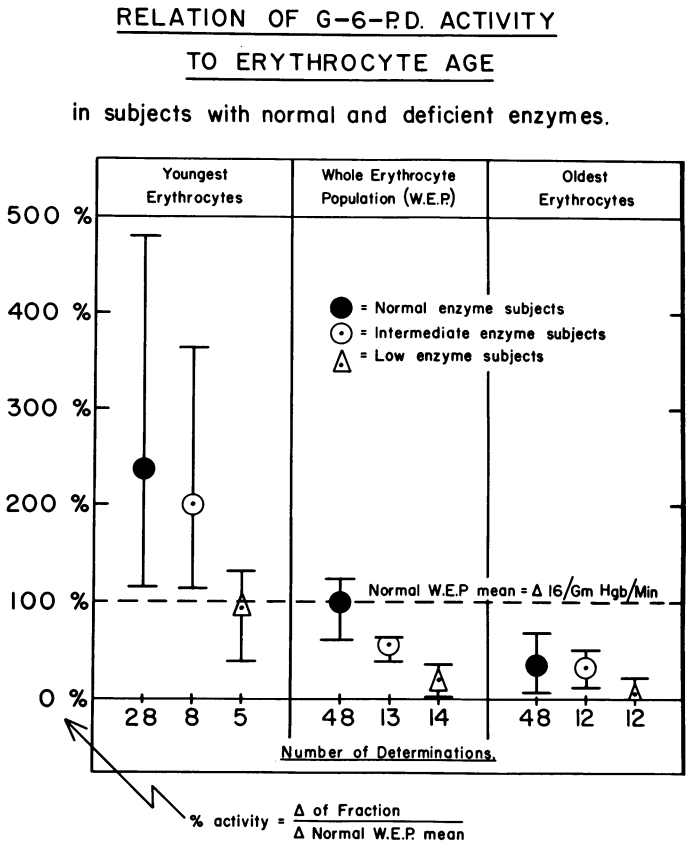

Fig. 2—The effect of age on the glucose-6-phosphate dehydrogenase activity of erythrocytes from subjects with normal, intermediate and low levels of dehydrogenase in the whole erythrocyte population. The mean level of enzyme for normal whole erythrocyte population is taken as equal to 100 per cent. The mean value plus and minus one standard deviation are represented for the whole erythrocyte population and youngest and oldest cell fractions for each of the three groups of subjects studied. The per cent activity is expressed as a per cent of the mean value for the normal whole erythrocyte population (Δ of fraction/ Δ normal W.E.P. mean). The methods employed to obtain these data are indicated in the text.

erythrocyte population was deficient in glucose-6-phosphate dehydrogenase, the level of this enzyme in young red cells, though not equal to normal levels, was distinctly higher than in old cells (Figure 2). No evidence was obtained for an inhibitor of glucose-6-phosphate dehydrogenase in hemolysates of erythrocytes deficient in this enzyme upon

TABLE I

INCIDENCE OF ERYTHROCYTE GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY IN A RANDOM POPULATION AND AMONG RELATIVES OF PROPOSITI

Random Population	*Subjects tested*	*% affected*
Total	666	7.3
Negroes	361	13.0
Caucasian	305	0.7
Family Studies		
Relatives of 31 propositi	154	40.0

incubation with hemolysates prepared from cells of normal subjects or with purified glucose-6-phosphate dehydrogenase[17, 32]. Thus, drug-induced hemolysis may cease when the older cells, deficient in dehydrogenase, have been destroyed. The remaining red cell population would be composed of young cells with higher levels of this enzyme and presumably relatively resistant to hemolysis. This hypothesis remains to be proved. It has been observed that following a drug-induced hemolytic episode, there is a rise in the level of erythrocyte glucose-6-phosphate dehydrogenase coincident with a reticulocytosis[30, 34, 35]. Such elevations in enzyme level have persisted for 40-80 days following the return of the reticulocyte count to within normal levels. Kimbro and co-workers[21] reported that readministration of nitrofurantoin at 35 and 75 days after the first course induced further hemolysis. However, Flanagan and associates[34] observed that in a single subject, given 30 milligrams of primaquine continuously for one year, no further detectable hemolysis occurred despite return of glucose-6-phosphate dehydrogenase to the pre-therapy low levels.

Genetic Studies. It is apparent that the identification of glucose-6-phosphate dehydrogenase deficiency and lowered concentrations of reduced glutathione as important defects in erythrocytes of drug-sensitive subjects has not as yet provided an explanation for the mechanism of their increased susceptibility to hemolysis. Nevertheless, they have provided important tools for studying the incidence and hereditary aspects of this "error" in erythrocyte metabolism.

In addition to the relatively high incidence of this defect among American Negroes[12-14, 36], it has been reported in Sephardic Jews[29],

TABLE II

SEX DISTRIBUTION OF SUBJECTS WITH GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENT RED CELLS[1]

	Middle[2]	*Low*[3]
Males	8	62
Females	48	3

Notes

1. This table includes subjects found to have erythrocytes deficient in glucose-6-phosphate dehydrogenase in the random population study and among relatives of propositi.
2. Middle refers to erythrocyte glucose-6-phosphate dehydrogenase activity between 2.5 and 4 standard deviations below the mean for the control group.
3. Low refers to erythrocyte glucose-6-phosphate dehydrogenase activity more than 4 standard deviations below the mean for the control group.

Italians[37, 38] primarily of Sicilian and Sardinian descent and subjects of Greek descent[13, 17]. In a random population of 666 subjects studied in the San Francisco and New York City areas, the observed incidence of erythrocyte glucose-6-phosphate dehydrogenase deficiency was 0.7 per cent among the Caucasians and 13.0 per cent among the Negroes (Table I). These findings are in general agreement with those of several other workers employing either the enzyme assay or the "glutathione stability test"[12, 14, 36, 39]. The bimodal distribution of this defect in a random population study suggests that the trait is genetically determined. An hereditary determination of this trait is strongly indicated by the finding of an incidence of 40 per cent among 154 relatives of 31 propositi (Table I). A similar incidence of affected relatives of drug-sensitive subjects was reported by Childs and co-workers[36] in the Baltimore area and by Szeinberg and Sheba[29] among Oriental Jews. Observations by several investigators are compatible with the trait being inherited as a sex-linked gene of intermediate dominance[13, 29, 36]. In families where the mother presented the abnormality, it was passed on to both the male and the female child, whereas affected fathers transmitted the defect only to daughters. Without exception, in 26 families studied to date, we have observed no incidence of a male with a low red cell dehydrogenase being born to a female with a normal level. Among parents of affected subjects there is a striking preponderance of mothers, 25 out of 29. These findings suggest that the trait is transmitted by a gene linked to the female sex chromosome.

TABLE III

DISTRIBUTION OF ERYTHROCYTE GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY AMONG A RANDOM POPULATION OF NEGROES

	Normal[1]	*Middle*[2]	*Low*[2]
Male[3]	177	0	17
Female	184	28	2

Notes

1. Erythrocyte glucose-6-phosphate dehydrogenase activity among normals was found to be 15.9 ± 2.4 change in optical density units per gram of hemoglobin[35].
2. See notes 1 and 2, Table II.
3. *Calculation of gene frequency*[39]:
   Assuming the trait is sex-linked, the genotype frequency (q) in the male population will be equal to the gene frequency. The frequency of the trait among female heterozygotes (2 pq) can be calculated from the observed incidence of the male hemizygote (q = .0971) in the male population (p = 1-q = .9029). This yields a calculated value for female heterozygotes (2 pq) in the above population equal to 17.5 per cent. The observed value was 16.3 per cent. The calculated and observed values are not significantly different (p = >> .05).

Subjects with low erythrocyte glucose-6-phosphate dehydrogenase may be divided into two groups on the basis of the severity of the enzymatic deficiency. Subjects with intermediate levels of enzyme (2.5 to 4 standard deviations below the mean for the control group) are predominantly affected females, whereas persons with low levels of enzyme (more than 4 standard deviations below the mean for the control group) are almost entirely males (Table II). It has been suggested[36] that the low and intermediate enzyme levels represent the homozygous and heterozygous states, respectively. However, we have observed in one family a mother with a very low level of glucose-6-phosphate dehydrogenase who gave birth to four normal sons. There are alternative explanations for the sex distribution of affected subjects. These include 1) that the phenotypic expression is modified in the female or 2) that we are dealing with a characteristic determined by more than a single genetic defect, i.e., a genetically heterogenous trait.

The frequency of the trait among heterozygous females may be predicted from calculations based on the incidence of male hemizygotes in a population[39]. In this calculation, the assumption is made that the trait is sex-linked. In a sex-linked trait, the genotype frequency among males will equal the gene frequency. Such a calculation (Table III, Note 3) indicates that there is excellent agreement between the pre-

TABLE IV

GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY IN LEUKOCYTES (WBC) AND RED CELLS (RBC) OF CONTROL SUBJECTS AND PERSONS WITH A DEFICIENCY OF THIS ENZYME IN THEIR CIRCULATING ERYTHROCYTES

Subjects	*Number*	*WBC**	*RBC*
Control	31	37.3 ± 5.8	0.49 ± 0.11
Deficient Red Cell G-6-P.D.**	14	36.8 ± 1.8	0.07 ± 0.01

Notes

*The unit of enzyme activity is expressed as the change in optical density per minute per 10^9 cells (Δ OD/min/10^9 cells). The mean values plus or minus one standard deviation are given in this table.

**G-6-P.D. is glucose-6-phosphate dehydrogenase. All subjects included in this group had erythrocyte glucose-6-phosphate dehydrogenase levels below three standard deviations of the mean for the control group.

dicted and actually observed incidence of low enzyme levels among females. This finding suggests that the enzyme assay provides a relatively accurate tool for the detection of the heterozygous state and, hence, the potential carrier of this defect.

The enzyme assay also affords a ready means of screening subjects for an increased susceptibility to drug-induced hemolytic anemias. Pertinent to the use of such an *in vitro* screening test are the observations of Alving and his co-workers[12] that the "reduced glutathione stability test" does not accurately reflect susceptibility to increased hemolysis following primaquine administration. Although we have found a high degree of correlation between the "glutathione stability test" and the level of glucose-6-phosphate dehydrogenase in erythrocytes, certain subjects were observed whose enzyme activity was decreased, yet whose *in vitro* glutathione stability test was within normal levels[13]. Further evaluation of erythrocyte glucose-6-phosphate dehydrogenase activity as an index of susceptibility to increased hemolysis must await studies correlating *in vitro* assays of red cell glucose-6-phosphate dehydrogenase with drug-induced hemolytic episodes.

Enzyme Activity in Tissues of Subjects with Glucose-6-Phosphate Dehydrogenase Deficient Erythrocytes. Glucose-6-phosphate dehydrogenase has been assayed in leukocytes of subjects whose erythrocytes are deficient in this enzyme[40]. The level of dehydrogenase in the leukocytes from these subjects did not differ from that in the white

cells of normal subjects (Table IV). In addition, a subject whose erythrocyte glucose-6-phosphate dehydrogenase was markedly deficient was not found to have a similar decrease in his liver enzyme activity. These findings are relevant to the question of the relationship between the genetic defect and the phenotypic expression which is measured as the level of glucose-6-phosphate dehydrogenase. If a suppression in the synthesis of this enzyme was the direct effect of the abnormal gene, all tissues of the affected subject would be expected to exhibit a similar enzyme deficiency. Accordingly, these data suggest that erythrocyte glucose-6-phosphate dehydrogenase is not a primary, but rather is a more remote consequence of the presence of a genetic defect.

Summary of Current Concepts of "Drug-Induced" Hemolytic Anemias:

1. Drug-induced hemolytic anemias are associated with certain intrinsic defects of erythrocyte metabolism, chief among which may be a deficiency in glucose-6-phosphate dehydrogenase.
2. Detection of susceptible individuals, as well as genetic carriers, is possible by assay *in vitro* of glucose-6-phosphate dehydrogenase levels in red cells and perhaps, with a lesser degree of accuracy, erythrocyte reduced glutathione stability.
3. The defect in erythrocyte metabolism seems to be determined genetically and may be transmitted as a sex-linked trait of intermediate dominance. The nature of the primary effect of the abnormal gene is unknown.
4. The mechanism of the hemolytic anemia remains to be elucidated.

Congenital Galactosemia

The genetically determined defect in the metabolism of the erythrocytes of subjects with galactosemia reflects an enzymatic deficiency, the consequences of which are not restricted to the erythrocyte, as may be the case for the defect in the drug-sensitive hemolytic anemias. Galactosemia is a congenital and hereditary disorder, characterized by difficulty in the metabolism of ingested galactose or milk. Ingestion of galactose by these patients is associated with the development of impaired function of the liver and spleen, of cataract formation, and of mental retardation[41]. The laboratory usually reveals marked galactosuria and an abnormal galactose tolerance test. As the disease pro-

gresses, hepatic insufficiency and evidence of renal functional impairment are frequent complications. The elimination of galactose from the diet may lead to improvement and even disappearance of symptoms.

Galactose Metabolism. The metabolism of galactose involves the following series of reactions[42, *]:

1) galactose + ATP $\xrightarrow{\text{galactokinase}}$ galactose-1-phosphate + ADP.

2) galactose-1-phosphate + UDPG $\xrightarrow[\text{urydil transferase}]{\text{galactose-1-phosphate}}$ UDPGal + glucose-1-phosphate.

3) UDPGal $\xrightarrow{\text{epimerase}}$ UDPG.

Galactose is phosphorylated to form galactose-1-phosphate in a reaction catalyzed by an enzyme called galactokinase and requiring, as a source of high energy phosphate, ATP. In reaction 2, galactose-1-phosphate is converted to glucose-1-phosphate. This reaction involves, as a co-factor, UDPG. The resulting glucose-1-phosphate is not derived directly from galactose-1-phosphate. The glucose-1-phosphate is formed from UDPG and galactose-1-phosphate becomes attached to the nucleotide resulting in UDPGal. The enzyme catalyzing this reaction is referred to as galactose-1-phosphate urydil transferase. In reaction 3, UDPGal may be directly converted to UDPG. The only structural difference between the two hexoses, galactose and glucose, is in the orientation of the hydrogen and hydroxyl groups about the fourth carbon atom. The mechanism of reaction 3 is believed to involve the removal of two hydrogen atoms from the fourth carbon atom of the hexose, presumably forming a ketone derivative. These two hydrogen atoms may then be returned to the hexose with the formation of the opposite compound or epimere, UDPG, when the starting compound is UDPGal. The enzyme catalyzing this reaction is called epimerase. The net result of this series of reactions is to permit the entrance of galactose into the general carbohydrate metabolism by converting it to glucose-1-phosphate and providing for the regeneration of the necessary co-factor, UDPG.

Enzyme Defect in Galactosemia. In 1956 Schwarz and his co-workers[43] reported that feeding of galactose or milk to infants with

* Abbreviations employed: ATP, adenosine triphosphate; UDPG, uridine diphosphate glucose; UDPGal, uridine diphosphate galactose; UTP, uridine triphosphate.

TABLE V

ENZYME ACTIVITY IN ERYTHROCYTES OF SUBJECTS WITH CONGENITAL GALACTOSEMIA[46]

Enzyme	*μ Moles/ml/hr.* *Normal*	*Galactosemia (12 cases)*
Galactokinase	0.12	0.10
Galactose-1-Phosphate Urydil Transferase	0.75	0.00
Epimerase	0.32	0.40

congenital galactosemia was associated with the accumulation of galactose-1-phosphate in their red cells. Such an accumulation did not occur in erythrocytes of normal subjects. These observations were confirmed and extended by Kalckar, Anderson and Isselbacher[44] who demonstrated that erythrocytes from galactosemic subjects incubated *in vitro* with galactose accumulated galactose-1-phosphate while red cells from normal subjects did not. These investigators suggested that the defect in galactosemia might reside in the enzyme catalyzing reaction 2, urydil transferase. A deficiency of this enzyme could account for the accumulation of galactose-1-phosphate following administration of galactose.

Employing specific assays for the three enzymes catalyzing reactions 1 through 3, it was demonstrated that only the enzyme, galactose-1-phosphate urydil transferase, was deficient in erythrocytes from galactosemic subjects compared to those of normal controls (Table V)[45].

Identification of this specific enzymatic defect in erythrocytes of galactosemic subjects provides a specific and relatively easy diagnostic test for this condition. The enzymatic assay avoids any hazard involved in the galactose tolerance test. In addition, it can be done at birth, on a small amount of blood.

Genetic Studies. Unlike the findings in subjects with erythrocyte glucose-6-phosphate dehydrogenase deficiency, two persons with congenital galactosemia have been demonstrated to lack the transferase in liver tissue in addition to red cells[47]. Thus, the present data are compatible with galactosemia being due to a hereditary defect in which the primary action of the gene may be a suppression of synthesis of galactose-1-phosphate urydil transferase.

The frequent occurrence of galactosemia among sibs and offspring of consanguineous matings together with its equal distribution in both sexes has suggested that galactosemia is probably transmitted as a single autosomal recessive gene. The disease is presumably clinically apparent only in the homozygous state. It would be expected that if galactosemia were inherited as a recessive gene, both parents should be heterozygous and although clinically asymptomatic might give evidence of a deficiency in galactose-1-phosphate urydil transferase. On the basis of both galactose tolerance tests[48] and assays for the enzyme[49, 50], it would appear that some parents of galactosemic children have a detectable impairment in galactose metabolism and others do not. This failure to detect impairment among some subjects who are presumably heterozygous for the galactosemic defect may merely reflect a degree of variation in the level of enzyme with a distribution curve among heterozygotes that overlaps, on the high side, that of normal subjects. It has also been suggested[49] that these findings might be due to the presence of a suppressor gene or the fact that more than one genetic defect may cause the clinical picture of congenital galactosemia.

Mechanism of Symptomatology and Improvement with Age. The demonstration of a deficiency in galactose-1-phosphate urydil transferase in patients with congenital galactosemia neither explains the mechanism of the varied symptomatology of this disease, nor the curious clinical observation that patients with congenital galactosemia generally improve with age. This improvement is associated with an increased ability to metabolize galactose, as reflected in an improvement in their galactose tolerance test[51].

Studies in animals suggest that the accumulation of galactose-1-phosphate may be toxic and lead to abnormal neurological manifestations[52]. In addition, there is evidence to indicate that the accumulation of galactose-1-phosphate may interfere with glucose utilization and lead to metabolic derangements which may have relevance to the cause of the clinical symptoms. Thus, galactose-1-phosphate has been found to inhibit *in vitro*, the enzymes, phosphoglucomutase[54, 55] and glucose-6-phosphatase[46]. It also has been found that mutants of Escherichia coli which accumulate galactose-1-phosphate due to lack of galactose-1-phosphate urydil transferase show retardation of growth in the presence of galactose plus glucose compared to their growth in the presence of glucose without galactose[53].

The improved galactose metabolism in patients associated with increased age, may be explained on the basis of the recent demonstration by Isselbacher[56] that there is an additional pathway of galactose metabolism which permits the incorporation of galactose into uridine diphosphate galactose and, in turn, to form glucose derivatives. This pathway involves the direct conversion of galactose-1-phosphate to uridine diphosphate galactose by reaction with the nucleotide, uridine triphosphate:

$$\text{UTP} + \text{galactose-1-phosphate} \xrightarrow{\text{UDPGal pyrophosphorylase}} \text{UDPGal} + \text{pyrophosphate.}$$

It has also been found[57] that UDPGal pyrophosphorylase is very low in fetal and neonatal tissues compared with adult tissues. Further, in normal tissue the activity of this enzyme is only about one-sixth of that of the galactose-1-phosphate urydil transferase. Thus, it would appear that in normal subjects the galactose-1-phosphate urydil transferase pathway is the one of primary importance. However, in galactosemic patients who appear to lack this enzyme, the observation that the alternate pathway of galactose metabolism may increase in activity associated with aging could explain the improvement in symptoms in galactosemic patients as they grow older.

Summary of Current Concepts of Galactosemia.

1. Galactosemia appears to be a congenital disease associated with a specific enzymatic defect, galactose-1-phosphate urydil transferase deficiency.
2. The identification of this specific enzyme defect in erythrocytes provides a simple diagnostic tool for the detection of this syndrome.
3. The symptomatology associated with galactosemia may be related to the accumulation of galactose-1-phosphate in the tissues.
4. The improvement in galactose tolerance test and symptomatology with age may be referable to the development and utilization of an alternate pathway of galactose metabolism.

Drug-induced hemolytic anemia and congenital galactosemia are only two examples of genetically determined defects in metabolism which are reflected in biochemical alterations in the readily accessible erythrocyte. Undoubtedly, this is a fertile field for further investigations in the area of "in-born" errors of metabolism.

REFERENCES

1. Earle, D. P., Jr., Bigelow, F. S., Zubrod, C. G. and Kane, C. A. Studies on the chemotherapy of the human malarias. IX. Effect of pamaquine on the blood cells of man, *J. clin. Invest. 27:* 121-29, 1948.
2. Hockwald, R. S., Arnold, J., Clayman, C. B. and Alving, A. S. Status of primaquine. 4. Toxicity of primaquine in Negroes, *J. Amer. med. Assoc. 149:*1568-70, 1952.
3. Dern, R. J., Beutler, E. and Alving, A. S. The hemolytic effect of primaquine. II. The natural course of the hemolytic anemia and the mechanism of its self-limited character, *J. Lab. clin. Med. 44:*171-76, 1954.
4. Beutler, E., Dern, R. J. and Alving, A. S. The hemolytic effect on primaquine. IV. The relationship of cell age to hemolysis, *J. Lab. clin. Med. 44:* 439-42, 1954.
5. Beutler, E., Dern, R. J. and Alving, A. S. The hemolytic effect of primaquine. III. A study of primaquine-sensitive erythrocytes, *J. Lab. clin. Med. 44:* 177-84, 1954.
6. Horecker, B. L. and Hiatt, H. H. Pathways of carbohydrate metabolism in normal and neoplastic cells, *New Engl. J. Med. 258:*177-84 and 225-32, 1958.
7. Marks, P. A. A newer pathway of carbohydrate metabolism; the pentose phosphate pathway, *Diabetes 5:*276-83, 1956.
8. Brin, M. and Yonemoto, R. H. Stimulation of the glucose oxidative pathway in human erythrocytes by methylene blue, *J. biol. Chem. 230:*307-17, 1958.
9. Johnson, A. B. and Marks, P. A. Glucose metabolism and oxygen consumption in normal and glucose-6-phosphate dehydrogenase deficient human erythrocytes, *Clinical Research 6:* 187-88, 1958 (abstr.).
10. Rall, T. W. and Lehninger, A. L. Glutathione reductase of animal tissues, *J. biol. Chem. 194:*119-30, 1952.
11. Beutler, E., Dern, R. J., Flanagan, C. L. and Alving, A. S. The hemolytic effect of primaquine. VII. Biochemical studies of drug-sensitive erythrocytes, *J. Lab. clin. Med. 45:*286-95, 1955.
12. Alving, A. S. and others. Biochemical and genetic aspects of primaquine-sensitive hemolytic anemia, *Ann. intern. Med. 49:*240-48, 1958.
13. Gross, R. T., Hurwitz, R. E. and Marks, P. A. An hereditary enzymatic defect in erythrocyte metabolism: glucose-6-phosphate dehydrogenase deficiency, *J. clin. Invest. 37:*1176-84, 1958.
14. Beutler, E. The glutathione instability of drug-sensitive red cells. A new method for the *in vitro* detection of drug sensitivity, *J. Lab. clin. Med. 49:* 84-95, 1957.
15. Carson, P. E., Flanagan, C. L., Ickes, C. E. and Alving, A. S. Enzymatic deficiency in primaquine-sensitive erythrocytes, *Science 124:*484-85, 1956.
16. Gross, R. T. and Marks, P. A. An hereditary enzymatic defect in red blood cells: its relation to certain drug-induced hemolytic anemias, *Ann. N.Y. Acad. Sci. 75:*106-09, 1958.
17. Zinkham, W. H., Lenkard, R. E., Jr. and Childs, B. A deficiency of glucose-6-phosphate dehydrogenase activity in erythrocytes from patients with favism, *Bull. Johns Hopk. Hosp. 102:*169-75, 1958.
18. Schrier, S. L. and others. The hemolytic effect of primaquine. IX. Enzymatic abnormalities in primaquine-sensitive erythrocytes, *J. Lab. clin. Med. 52:* 109-17, 1958.
19. Schrier, S. L. and Kellermeyer, R. W. Compensatory mechanisms in primaquine-sensitive erythrocytes, *J. clin. Invest. 37:*928-29, 1958 (abstr.).
20. Schrier, S. L., Kellermeyer, R. W. and Alving, A. S. Coenzyme studies in primaquine-sensitive erythrocytes, *Proc. Soc. exp. Biol. Med. 99:*354-56, 1958.
21. Kimbro, E. L., Jr., Sachs, M. V. and Torbert, J. V. Mechanism of the hemolytic anemia induced by nitrofurantoin (Furadantin), *Bull. Johns Hopk. Hosp. 101:*245-57, 1957.
22. Dern, R. J., Beutler, E. and Alving,

A. S. The hemolytic effect of primaquine. V. Primaquine sensitivity as a manifestation of a multiple drug sensitivity, *J. Lab. clin. Med. 45*:30-39, 1955.

23. Szeinberg, A., Asher, Y. and Sheba, C. Studies on glutathione stability in erythrocytes of cases with past history of favism or sulfa-drug-induced hemolysis, *Blood 13*:348-58, 1958.
24. Zinkham, W. H. and Childs, B. Effect of vitamin K and naphthalene metabolites on glutathione metabolism of erythrocytes from normal newborns and patients with naphthalene hemolytic anemia, *A.M.A. J. Dis. Child. 94*:420-23, 1957.
25. Zinkham, W. H. and Childs, B. A defect of glutathione metabolism in erythrocytes from patients with a naphthalene-induced hemolytic anemia, *Pediatrics 22*:461-71, 1958.
26. Dawson, J. P., Thayer, W. W. and Desforges, J. F. Acute hemolytic anemia in the newborn infant due to naphthalene poisoning: report of two cases, with investigations into the mechanism of the disease, *Blood 13*:1113-25, 1958.
27. Szeinberg, A., Sheba, C., Hirshorn, M. and Bodonyi, E. Studies on erythrocytes in cases with past history of favism and drug-induced acute hemolytic anemia, *Blood 12*:603-13, 1957.
28. Sansone, G. and Segni, G. Sensitivity to broad beans, *Lancet 2A*:295, 1957.
29. Szeinberg, A. and Sheba, C. Hemolytic trait in oriental Jews connected with an hereditary enzymatic abnormality of erythrocytes, *Israel med. J. 17*:158-68, 1958.
30. Marks, P. A. Unpublished observations.
31. Marks, P. A., Johnson, A. B. and Hirschberg, E. Effect of age on the enzyme activity in erythrocytes, *Proc. nat. Acad. Sci. 44*:529-36, 1958.
32. Gross, R. T. and others. Studies on the hereditary enzymatic defect in certain drug induced hemolytic anemias, *Proc. Soc. Pediatric Research*, 26th Annual Meeting, 1958, p. 8 (abstr.). Manuscript in preparation.
33. Marks, P. A. and Johnson, A. B. Relationship between the age of human erythrocytes and their osmotic resistance: a basis for separating young and old erythrocytes, *J. clin. Invest. 37*:1542-48, 1958.
34. Flanagan, C. L., Schrier, S. L., Carson, P. E. and Alving, A. S. The hemolytic effect of primaquine. VIII. The effect of drug administration on parameters of primaquine sensitivity, *J. Lab. clin. Med. 51*:600-08, 1958.
35. Marks, P. A. Red cell glucose-6-phosphate and 6-phosphogluconic dehydrogenases and nucleoside phosphorylase, *Science 127*:1338-39, 1958.
36. Childs, B. and others. A genetic study of a defect in glutathione metabolism of the erythrocyte, *Bull. Johns Hopk. Hosp. 103*:21-37, 1958.
37. Sansone, G. and Segni, G. Prime determinazioni del glutatione (GSH) ematico nel favismo, *Boll. Soc. ital. Biol. Sper. 32*:456-58, 1956.
38. Panizon, F. Sul compartamento delle emazie dei fabici di fronte all' idrossilamina *in vitro*, *Studi sassaresi 35*: 164, 1957.
39. Motulsky, A. G. and Campbell, J. M. A screening test for glucose-6-phosphate dehydrogenase deficiency of the red cell suitable for genetic surveys. Possible relationship of the enzyme defect to malaria, *Blood*. In press.
40. Marks, P. A., Gross, R. T. and Hurwitz, R. E. Studies on the gene action in erythrocyte glucose-6-phosphate dehydrogenase deficiency: tissue enzyme levels, *Nature*. In press.
41. Mason, H. H. and Turner, M. E. Chronic galactemia, *Amer. J. Dis. Child. 50*:359-74, 1935.
42. Kalckar, H. M. and Maxwell, E. S. Biosynthesis and metabolic function of uridine diphosphoglucose in mammalian organisms and its relevance to certain inborn errors, *Physiol. Rev. 38*:77-90, 1958.
43. Schwarz, V., Golberg, L., Komrower, G. M. and Holzel, A. Some disturbances of erythrocyte metabolism in galactosaemia, *Biochem. J. 62*:34-40, 1956.
44. Kalckar, H. M., Anderson, E. P. and Isselbacher, K. J. Galactosemia, a congenital defect in nucleotide transferase:

a preliminary report, *Proc. nat. Acad. Sci., Wash. 42*:49-51, 1956.

45. Isselbacher, K. J., Anderson, E. P., Kurahashi, K. and Kalckar, H. M. Congenital galactosemia, a single enzymatic block in galactose metabolism, *Science 123*:635-36, 1956.
46. Isselbacher, K. J. Personal communication.
47. Anderson, E. P., Kalckar, H. M. and Isselbacher, K. J. Defect in uptake of galactose-1-phosphate into liver nucleotides in congenital galactosemia, *Science 125*:113-14, 1957.
48. Holzel, A. and Komrower, G. M. A study of the genetics of galactosaemia, *Arch. Dis. Childh. 30*:155-59, 1955.
49. Kirkman, H. N. and Kalckar, H. M. Enzymatic deficiency in congenital galactosemia and its heterozygous carriers, *Ann. N. Y. Acad. Sci. 75*:274-78, 1958.
50. Hsia, D. Y.-Y., Huang, I. and Driscoll, S. G. The heterozygous carrier in galactosemia, *Nature 182*:1389-90, 1958.
51. Townsend, E. H., Jr., Mason, H. H. and Strong, R. R. Galactosemia and its relation to Laennec's cirrhosis, *Pediatrics 7*:760-73, 1951.
52. Dam, H. Galactose poisoning in chicks, *Proc. Soc. exp. Biol. Med. 55*:57-59, 1944.
53. Kurahashi, K. and Wahba, A. J. Interference with growth of certain *Escherichia coli* mutants by galactose, *Biochim. biophys. Acta 30*:298-302, 1958.
54. Ginsburg, V. and Neufeld, E. F. Inhibition of phosphoglucomutase by galactose-1-phosphate. A possible factor in galactose toxicity, *Amer. Chem. Soc. Mtg.* Sept. 1957, 27 C (abstr.).
55. Sidbury, J. B. Inhibition of phosphoglucomutase with galactose phosphate, *Amer. Chem. Soc. Mtg.* Sept. 1957, 27C-28C (abstr.).
56. Isselbacher, K. J. A mammalian uridinediphosphate galactose pyrophosphorylase, *J. biol. Chem. 232*:429-44, 1958.
57. Isselbacher, K. J. Evidence for an accessory pathway of galactose metabolism in mammalian liver, *Science 126*: 652-53, 1957.